

Original Article

KDM5B is overexpressed in gastric cancer and is required for gastric cancer cell proliferation and metastasis

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Abstract: Epigenetic alterations such as aberrant expression of histone-modifying enzymes have been implicated in tumorigenesis. KDM5B (also known as JARID1B) is a newly identified histone demethylase that regulates chromatin structure or gene expression by removing methyl residues from trimethylated lysine 4 on histone H3. Recent observations have shown oncogenic activity of KDM5B. However, the role of KDM5B in gastric cancer carcinogenesis remains unclear. In this study, we aimed to investigate the role of KDM5B in gastric cancer. Immunohistochemical analysis, western blotting, and qRT-PCR were used to measure the levels of KDM5B in gastric cancer cell lines, 45 pairs of gastric cancer tissues and the adjacent nonneoplastic tissues. KDM5B and shKDM5B were transfected into gastric cancer cells to investigate its role on regulating cell proliferation which was measured by MTT and colony formation assay. Cell's migration and invasion were measured by Transwell and Matrigel analysis in vitro. PCNA expression was measured by immunofluorescence staining and immunohistochemical analysis. The in vivo tumorigenesis and metastasis assays were performed in SCID mice. In clinical gastric cancer samples, we found that KDM5B expression was significantly up-regulated in cancer lesions compared with paired normal gastric tissues. By silencing or overexpressing KDM5B in gastric cancer cells, we found that KDM5B could promote cell growth and metastasis in vitro. An in vivo assay showed that KDM5B not only dramatically promoted gastric cancer cell xenograft formation and growth but also promoted gastric cancer cell metastasis in a liver metastasis model. Moreover, we demonstrated that KDM5B promoted gastric cancer metastasis via regulation of the Akt pathway. Our study provided evidence that KDM5B functions as a novel tumor oncogene in gastric cancer and may be a potential therapeutic target for gastric cancer management.

Keywords: KDM5B, gastric cancer, proliferation, metastasis

Introduction

Gastric cancer is a malignancy with high incidence and the second leading cause of cancer death worldwide [1]. Numerous studies indicate that the development and progression of gastric cancer arises via miss-regulation of many related genes such as p53, p21, Akt, Gli1, and PTEN [2-7]. However, the regulatory mechanisms remain poorly understood. Therefore, discovery of critical carcinogenic pathways may be beneficial for the identification of new therapeutic targets for gastric cancer.

Epigenetic regulation also plays a critical role in the pathogenesis of gastric cancer [8, 9]. DNA methylation is a component of the epigenetic gene-silencing complex, whereas histone (H3 and H4) post-translational modifications comprise a ubiquitous component of rapid epigenetic changes [10-12]. Epigenetic changes are associated with altered transcription. Metastasis correlates with the loss of epithelial differentiation, induction of epithelial mesenchymal transition and the acquisition of a migratory phenotype, which are controlled by epigenetic alterations caused by the dysregulation of the

transcriptome in gastric cancer [13, 14]. Histone methylation/demethylation generally deactivates and activates genes by controlling the access of transcription factors to DNA [11]. Histone dysregulation caused by genetic and epigenetic alterations is a hallmark of cancer [15]. KDM5B-mediated histone H3K4 demethylation contributes to the silencing of retinoblastoma target genes in senescent cells, presumably by compacting chromatin and silencing certain genes [16]. Previous studies have found that KDM5B depletion stimulated p16 transcription and suppressed tumor cell growth in vitro and in vivo [17], suggesting that it plays a role in cell growth regulation in human cancer.

In the present study, we aimed to explore the tumor oncogene function of KDM5B in gastric cancer. We found that the expression of KDM5B was significantly up-regulated in human gastric cancer lesions compared with paired normal gastric tissues. We also demonstrated that KDM5B could promote the growth and metastasis of gastric cancer cell lines, both in vitro and in vivo. Our present manuscript suggests that KDM5B acts as a potential oncogene in gastric cancer.

Methods

Patients and tissue samples

A total of 45 gastric cancer tissue samples, along with matched normal gastric tissues, were used in this study. All of the samples were obtained from the department of gastrointestinal surgery, affiliated Hospital of Guilin Medical University between 2011 and 2013. For all of the patients who participated in this study, written informed consent was obtained, which was approved by the Ethical Committee of Guilin Medical University.

Cell culture and reagents

The gastric cancer cell lines (AGS, MKN45, SUN1, KATOIII, MKN28, NCI-N87, SUN16, GES-1, and BGC-823) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Antibodies that had been raised against Akt and phosphorylated Akt (Ser 473 and Thr 308) were purchased from Cell Signaling Technology (Beverly, MA, USA), and a mouse anti-KDM5B antibody was obtained from Abcam (Cambridge, MA, USA). The Akt

inhibitor LY294002, signal silence Akt siRNA and its control siRNA were purchased from Cell Signaling Technology. All of the remaining reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise specified.

Plasmid construction and transfection

For overexpression, the cDNA representing the complete open reading frame of KDM5B was cloned into the pBabe vector to generate the KDM5B expression plasmid. The expression plasmid was verified by sequencing both strands and was used to transfect the NCI-N87 cells to establish the KDM5B overexpression cell line. For KDM5B RNA interference, the control (pSuper) and pSuper-KDM5B shRNA plasmids were purchased from OligoEngine Biotechnology (Seattle, USA) and was used to transfect the GES-1 cells to establish the KDM5B knockdown cell line. The transfection efficiency of KDM5B was confirmed by western blotting and quantitative reverse transcription PCR (qRT-PCR) analyses.

MTT assay

A 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium (MTT) assay was used to assess cell proliferation. The cells were seeded and 20 μ l of the MTT solution (5 mg/ml) was then added to each well at the indicated time. The absorbance at 490 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

Colony formation assay

The cells were seeded in 6-cm dishes at a density of 300 cells per dish. After incubation for 14 days, the colonies were fixed with methanol for 10 min and stained with crystal violet for 15 min, after which point the number of colonies containing more than 50 cells was scored.

Western blot assay

Equal amounts of protein were separated using SDS polyacrylamide gels and were electrotransferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). The membranes were immunoblotted overnight at 4°C with primary antibodies, followed by their respective secondary antibodies. β -Actin was used as the loading control.

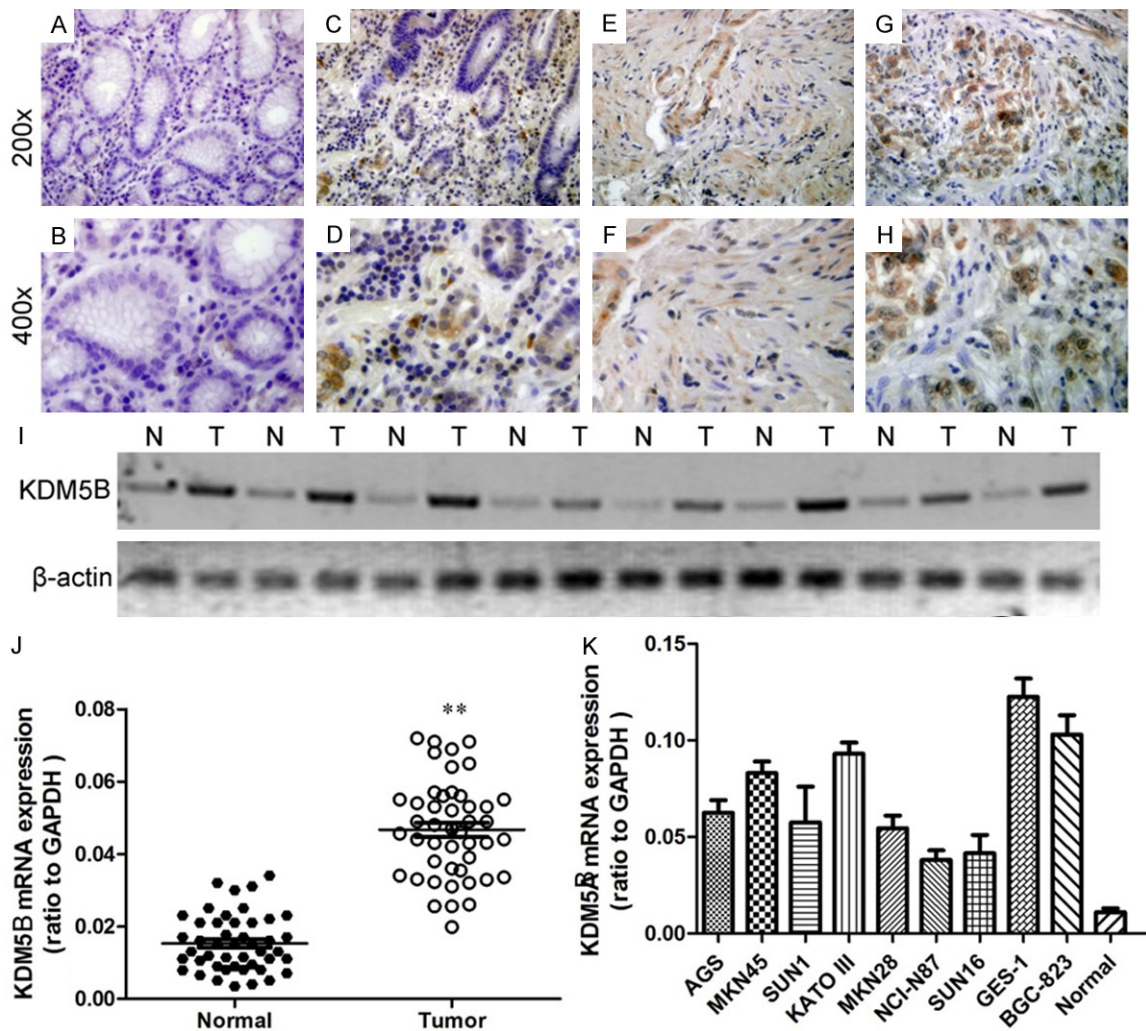


Figure 1. Expression of KDM5B in gastric cancer tissues and gastric cancer cell lines. Immunohistochemistry analysis showed KDM5B expression in paraffin-embedded sections from normal gastric tissues (A, B) and gastric cancer tissues (C-H: C, D, Weak staining in gastric cancer; E, F, Moderate staining in gastric cancer. G, H, Strong staining in gastric cancer). (I) KDM5B protein levels in tumor tissues and matched normal tissue lesions, as assessed using western blot analyses. T: gastric cancer tissues; N: normal gastric tissues. (J) KDM5B mRNA levels in tumor tissues and matched normal tissue lesions, as assessed using qRT-PCR analyses. (K) KDM5B mRNA levels in gastric cancer cell lines and normal tissue were assessed by qRT-PCR. $^{**}P < 0.01$ is based on Student's *t*-test. All results are from three or four independent experiments. Error bars indicate standard deviation.

Quantitative reverse-transcription PCR

RNA was extracted using TRIzol reagent, according to the manufacturer's recommended protocol (Invitrogen). qRT-PCR was performed using Applied Biosystems (Foster City, CA, USA) StepOne and StepOne Plus Real-Time PCR Systems. GAPDH was used as a loading control. The experiments were repeated a minimum of three times to confirm the results.

Immunofluorescence staining

The cells were grown on the sterile coverslips, and the cells were fixed with 4% paraformaldehyde and permeabilized using 0.1% Triton-X100.

Cells were blocked with rabbit anti-PCNA antibody followed by horseradish peroxidase-conjugated anti-rabbit secondary antibody. Finally, the cells were further stained with 4, 6-diamidino-2-phenylindole (DAPI).

Chamber assay

Migration and invasion assays were performed as previously described [18]. Invasion assays were performed in 24-well transwell chambers (BD Biosciences, Bedford, MA, USA) containing polycarbonate filters with 8-mm pores coated

Table 1. KDM5B staining and clinicopathologic characteristics of 236 gastric cancer patients

Variables	KDM5B staining		Total	P ^a
	Low	High		
Age (y)				
≤ 65	45 (38%)	72 (62%)	117	0.184
> 65	39 (33%)	80 (67%)	119	
Sex				
Male	65 (35%)	117 (65%)	182	0.119
Female	19 (30%)	45 (70%)	64	
Tumor diameter (cm)				
≤ 5	47 (37%)	80 (63%)	127	0.355
> 5	37 (34%)	72 (66%)	109	
Differentiation				
Well	4 (57%)	3 (43%)	7	0.261
Moderate	49 (36%)	89 (64%)	138	
Poor	25 (32%)	53 (68%)	78	
Unknown ^b	6 (46%)	7 (54%)	13	
Depth of invasion				
T1	13 (45%)	16 (55%)	29	0.027 ^c
T2	16 (46%)	19 (54%)	35	
T3	51 (34%)	100 (66%)	151	
T4	4 (19%)	17 (81%)	21	
Lymph node metastasis				
N0	39 (38%)	65 (62%)	104	0.041
N1/N2/N3	45 (34%)	87 (66%)	132	
Distant metastasis				
M0	23 (44%)	29 (56%)	52	0.039
M1	61 (33%)	123 (67%)	184	
TNM stage				
I	14 (42%)	19 (58%)	33	0.024 ^d
II	25 (46%)	29 (54%)	54	
III	41 (34%)	78 (66%)	119	
IV	4 (20%)	16 (80%)	20	

^aX² test. ^bUnknown: unspecified subtypes. ^cComparing the depths of invasion T1-T3 versus T4. ^dComparing TNM stages I-II versus III-IV.

with Matrigel (BD Biosciences). First, the cells that were suspended in serum-free DMEM were added to the upper compartment of the chamber and medium containing 10% FBS was added to the lower compartment of the chamber. At the indicated timepoints, the number of cells that had migrated through the membrane and attached to the lower surface of the membrane was counted under a light microscope for a minimum of ten random visual fields. The migration assay was similar to the migration assay except that the upper side of the membranes was not coated with the Matrigel.

In vivo tumorigenesis and metastasis assays

The in vivo tumorigenesis and metastasis assays were performed, as previously described [18]. Briefly, 1×10^6 cells were injected subcutaneously into the right flanks of severe combined immunodeficient (SCID) mice. Tumor length (L) and width (W) were measured every 3 days, and tumor volume was calculated using the equation: volume = $(W^2 \times L)/2$. After 6 weeks, the mice were killed and the tumor volume and weight were measured. To produce the lung metastasis model, 5×10^5 cells were injected into the lateral tail veins of female SCID mice. After 6 weeks, the mice were killed and the liver tissues were harvested for use in further experiments. All of the animal experiments were performed with the approval of the Guilin Medical University Animal Care and Use Committee.

Histological and immunohistochemical analysis

The tumors and livers were fixed in 4% paraformaldehyde in phosphate-buffered saline overnight and subsequently embedded in paraffin wax. Sections were cut at a thickness of 4 μ m and stained with hematoxylin and eosin for histological analysis. Immunohistochemical analysis was also performed for KDM5B and PCNA in tumors from different types of cells as described previously [18].

Statistical analysis

The results were analyzed using SPSS 18.0 software (Chicago, IL, USA). Each experiment was repeated a minimum of three times. A two-tailed t-test was used to determine statistical significance. The results were presented as the means \pm S.D. P-values < 0.05 were considered to be statistically significant.

Results

Expression of KDM5B was upregulated in gastric cancer tissues

To investigate whether KDM5B might be involved in gastric carcinogenesis, the expression level of KDM5B in gastric cancer tissues

KDM5B promotes gastric cancer cell proliferation and metastasis

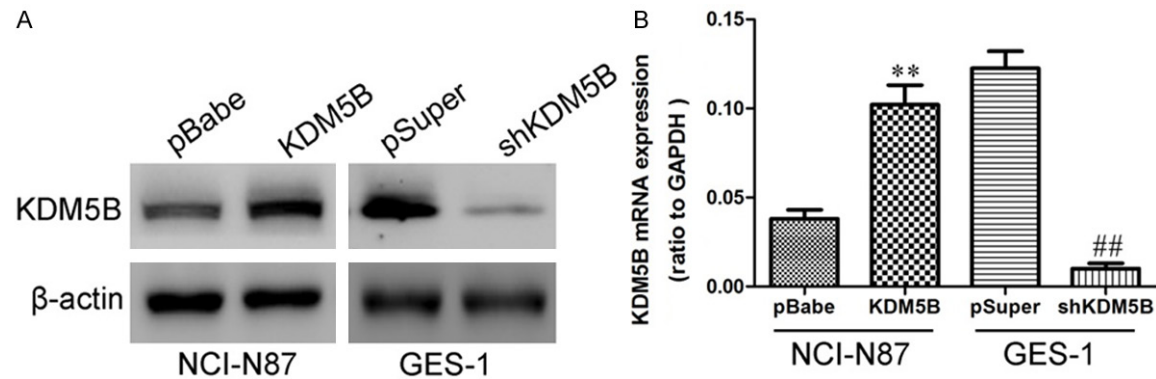


Figure 2. Transfection efficiency of KDM5B in gastric cancer cell lines. A. The transfection efficiency of KDM5B in NCI-N87 and GES-1 cells was analyzed by measuring protein levels by western blotting. β -actin was used as a loading control. B. The transfection efficiency of KDM5B in NCI-N87 and GES-1 cells was analyzed by measuring mRNA levels by qRT-PCR analyses. The data represent the means \pm S.D. of three independent experiments. ** $P < 0.01$; ## $P < 0.01$.

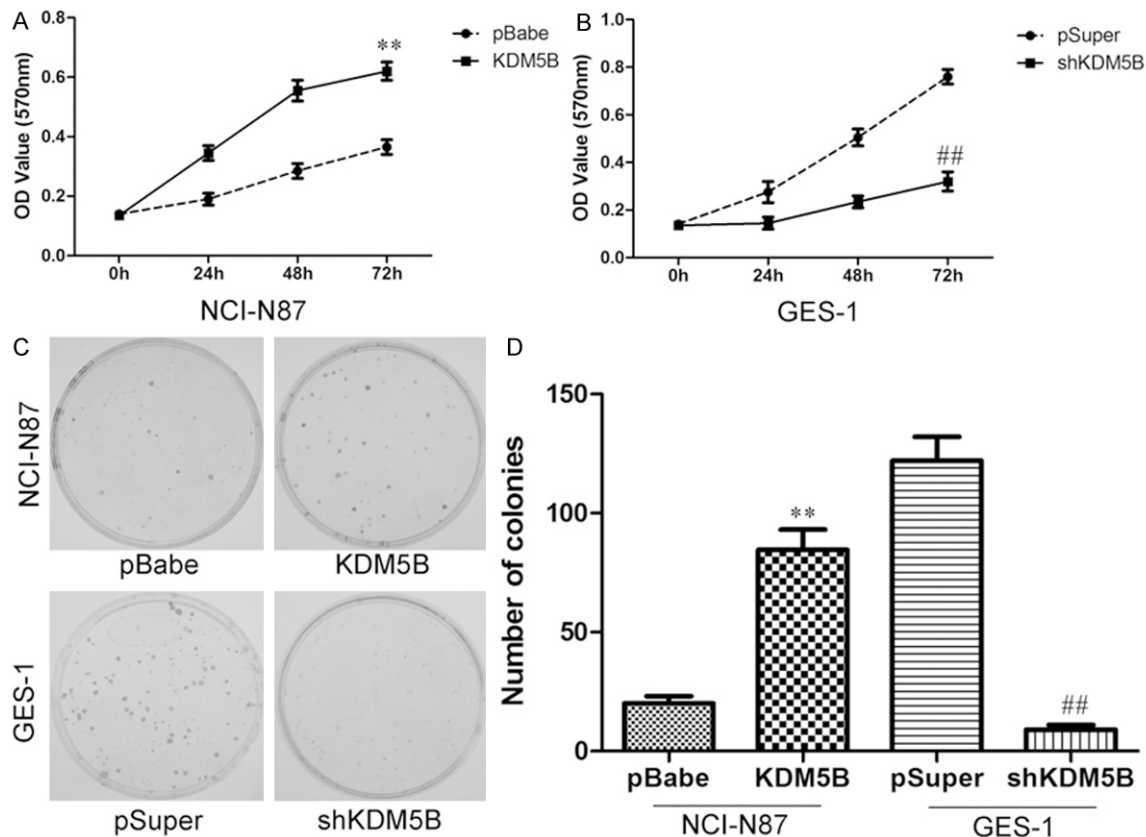


Figure 3. KDM5B promoted gastric cancer cell proliferation. A. Cell proliferation after KDM5B overexpression in NCI-N87 cells was measured using MTT assays. B. Cell proliferation after KDM5B knockdown in GES-1 cells was measured using MTT assays. C. The results of colony formation assays that were conducted after KDM5B overexpression in NCI-N87 cells and knockdown in GES-1 cells. D. The summary graphs are presented for the colony formation assay that was outlined in C. The data represent the means \pm S.D. of three independent experiments ** $P < 0.01$.

and its matched normal gastric tissues was determined by immunohistochemistry using a KDM5B specific antibody. As compared with

normal gastric tissues, gastric cancer specimens showed overexpression of KDM5B (Figure 1A-H). The protein and mRNA levels of

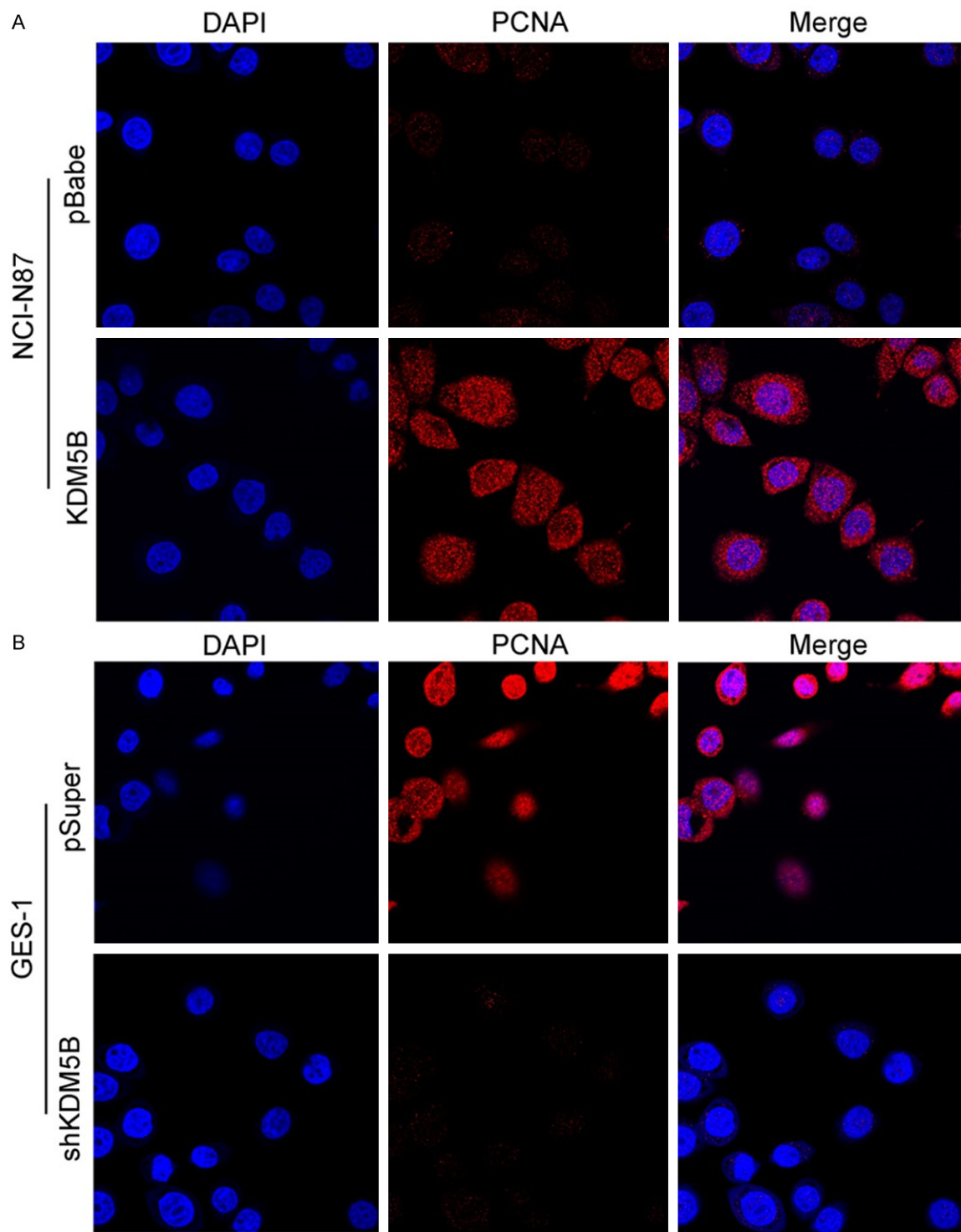


Figure 4. Effects of KDM5B on PCNA in gastric cancer cell lines. A. Immunofluorescence staining of PCNA in NCI-N87-transfected cell lines. B. Immunofluorescence staining of PCNA in GES-1-transfected cell lines.

KDM5B in these tissue samples were also analyzed by western blot (**Figure 1I**) and qRT-PCR (**Figure 1J**), respectively. The protein and mRNA levels of KDM5B were upregulated in gastric

cancer samples as compared with the normal gastric samples. Furthermore, we measured the mRNA expression levels of KDM5B in the nine gastric cancer cell lines and a normal gas-

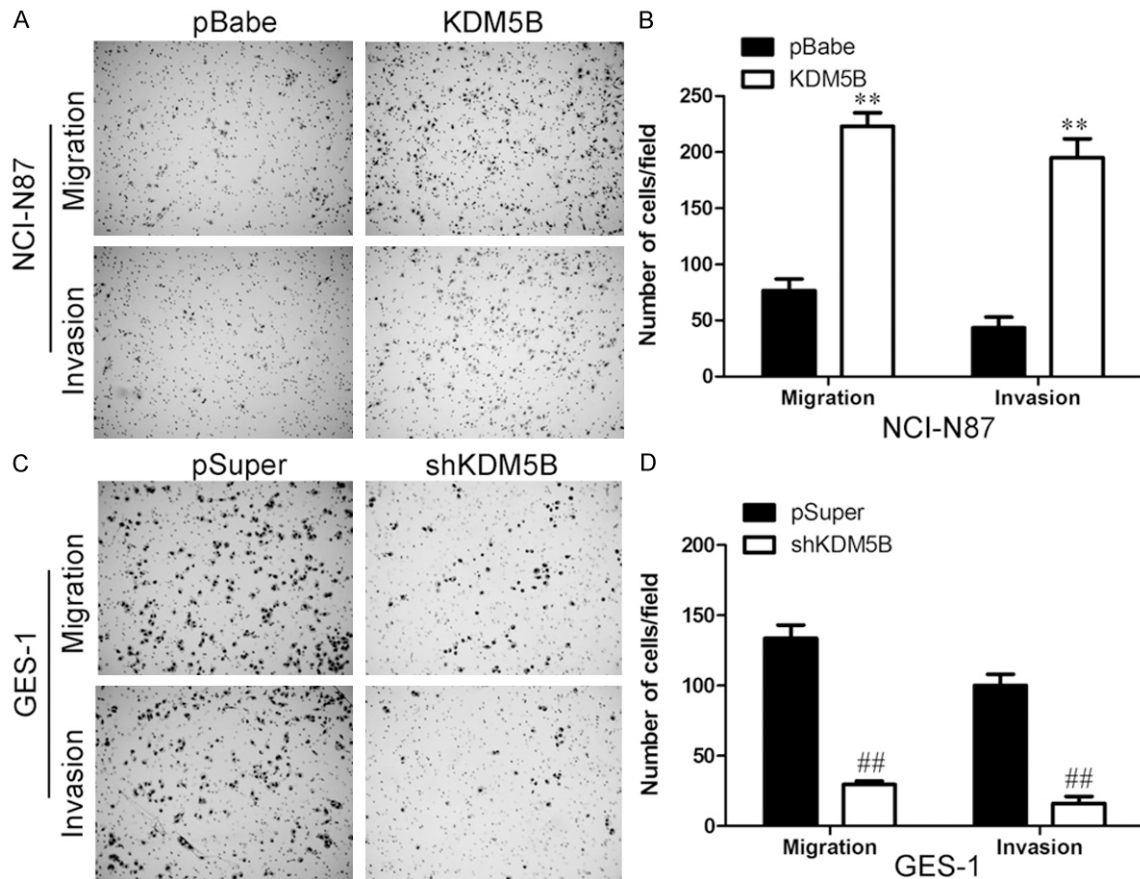


Figure 5. KDM5B inhibited gastric cancer cell mobility. A. Migration and invasion ability was measured using Transwell and Matrigel assays in KDM5B-transfected NCI-N87 cells. B. The summary graphs are presented for the experiment that was outlined in A. C. Migration and invasion ability was measured using Transwell and Matrigel assays in shKDM5B-transfected GES-1 cells. D. The summary graphs are presented for the experiment that was outlined in C. The data represent the mean number of cells per field and are presented as the means \pm S.D. ** $P < 0.01$.

tric tissue by qRT-PCR. As showed in **Figure 1K**, expression level of KDM5B mRNA in gastric cancer cell lines was higher than that in the normal gastric tissue. Significant correlations were found between KDM5B expression and depth of invasion ($P = 0.027$), TNM stage ($P = 0.024$) (**Table 1**). There were no statistical connections between KDM5B expression and the rest clinicopathological parameters, such as patient age, gender, and tumor size. These data demonstrated that the upregulation of KDM5B might be relevant to development of gastric cancer.

Establishment of stable KDM5B transfectants in gastric cancer cell lines

As showed in **Figure 1K** NCI-N87 had the lowest expression level of KDM5B among the nine gastric cell lines. So, we used NCI-N87 cells to

establish a stable cell line that constitutively overexpressed the KDM5B protein with the aim of revealing the role that KDM5B expression has in the development or progression of gastric cancer. We also used shRNA to generate a stable KDM5B knockdown in the GES-1gastric cancer cell line, which had a high KDM5B expression. The transfection efficiency was confirmed using western blotting and qRT-PCR analyses. As shown in **Figure 2A** and **2B**, the NCI-N87 cells that had been transfected with the KDM5B expression plasmid displayed significantly increased KDM5B expression at both the mRNA and protein levels compared with the vector cell lines. In addition, the GES-1 cells that had been transfected with the KDM5B shRNA plasmid displayed significantly decreased KDM5B expression at both the mRNA and protein levels compared with the control cells.

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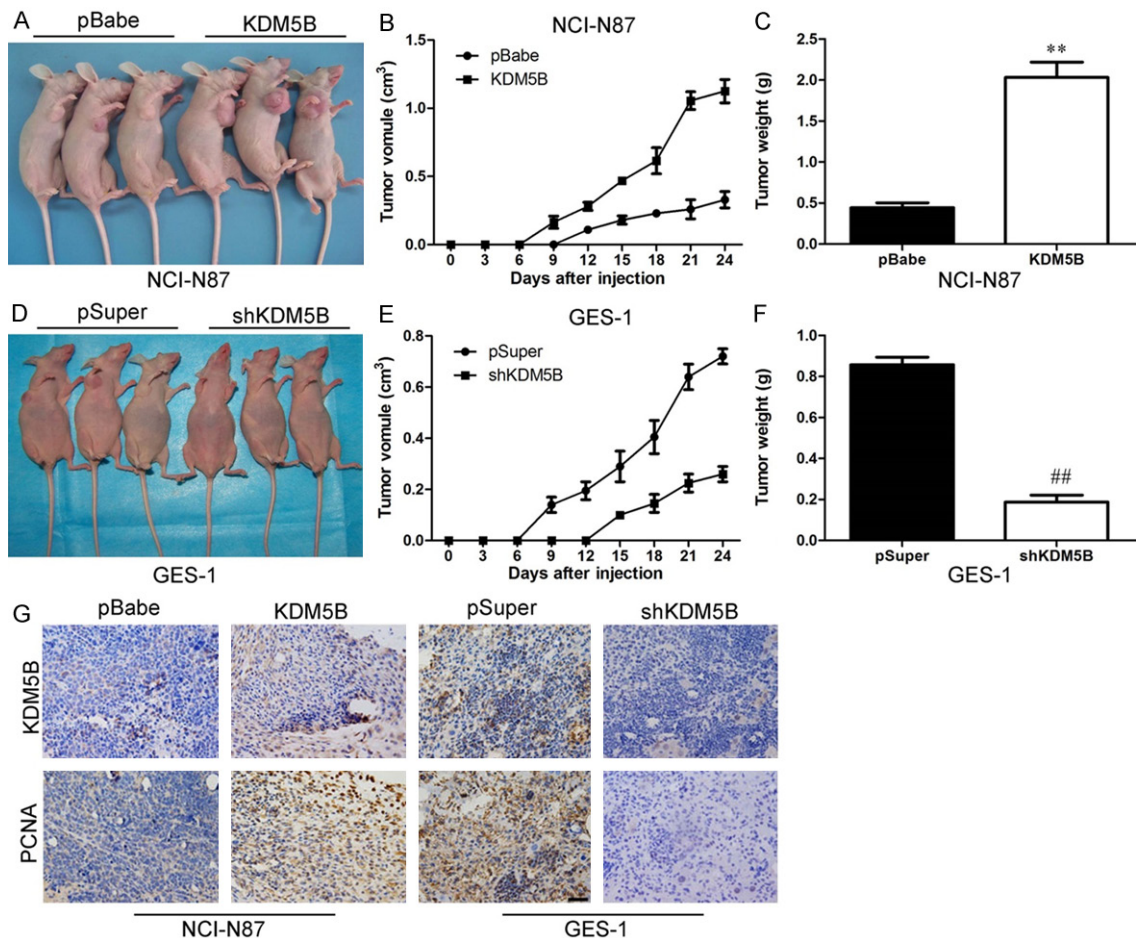


Figure 6. KDM5B promoted tumorigenesis in vivo. A. Stable KDM5B-overexpressing NCI-N87 and its control cells were inoculated subcutaneously into the axilla of six male BALB/*c-nu/nu* mice. B. Growth curves of mammary tumor value after the injection of KDM5B-overexpressing NCI-N87 and its control cells into SCID mice. The error bars represent the means \pm S.D. ($n = 6$). C. Tumor weight was measured. D. Stable KDM5B-silenced GES-1 and its control cells were inoculated subcutaneously into the axilla of six male BALB/*c-nu/nu* mice. E. Growth curves of mammary tumors after the injection of KDM5B-silenced GES-1 and control cells into SCID mice. The error bars represent the means \pm S.D. ($n = 6$). F. Tumor weight was measured. G. The KDM5B and PCNA protein expression were measured by immunohistochemistry staining in tumor tissues. $**P < 0.01$; $##P < 0.01$.

KDM5B promoted gastric cancer cell proliferation

We first explored the effects of KDM5B expression on cell growth using the MTT assay. As shown in **Figure 3A**, KDM5B overexpression significantly enhanced the growth of NCI-N87 cells, whereas KDM5B knockdown significantly inhibited the growth of GES-1 cells (**Figure 3B**). Next, we performed a clonogenic assay to confirm the effects of KDM5B on proliferation. We found that KDM5B overexpression dramatically increased the colony formation efficiency of NCI-N87 cells, whereas the colony formation efficiency was dramatically reduced in the GES-1 shRNA cell lines (**Figure 3C** and **3D**). As PCNA is an important marker of cell proliferation,

we next examined the PCNA by immunofluorescence staining. As shown in **Figure 4**, we found that the overexpression of KDM5B in NCI-N87 cells significantly upregulated PCNA staining (**Figure 4A**). Also knockdown of KDM5B in GES-1 cells dramatically downregulated the staining of PCNA (**Figure 4B**). These results suggested that KDM5B could significantly promote the proliferation of gastric cancer cells.

KDM5B promoted gastric cancer cell migration and invasion

We next assessed whether KDM5B could affect the ability of gastric cancer cells to migrate and invade using a transwell assay. KDM5B overexpression promoted both migration and invasion in NCI-N87 cells (**Figure 5A** and **5B**). In addition,

KDM5B promotes gastric cancer cell proliferation and metastasis

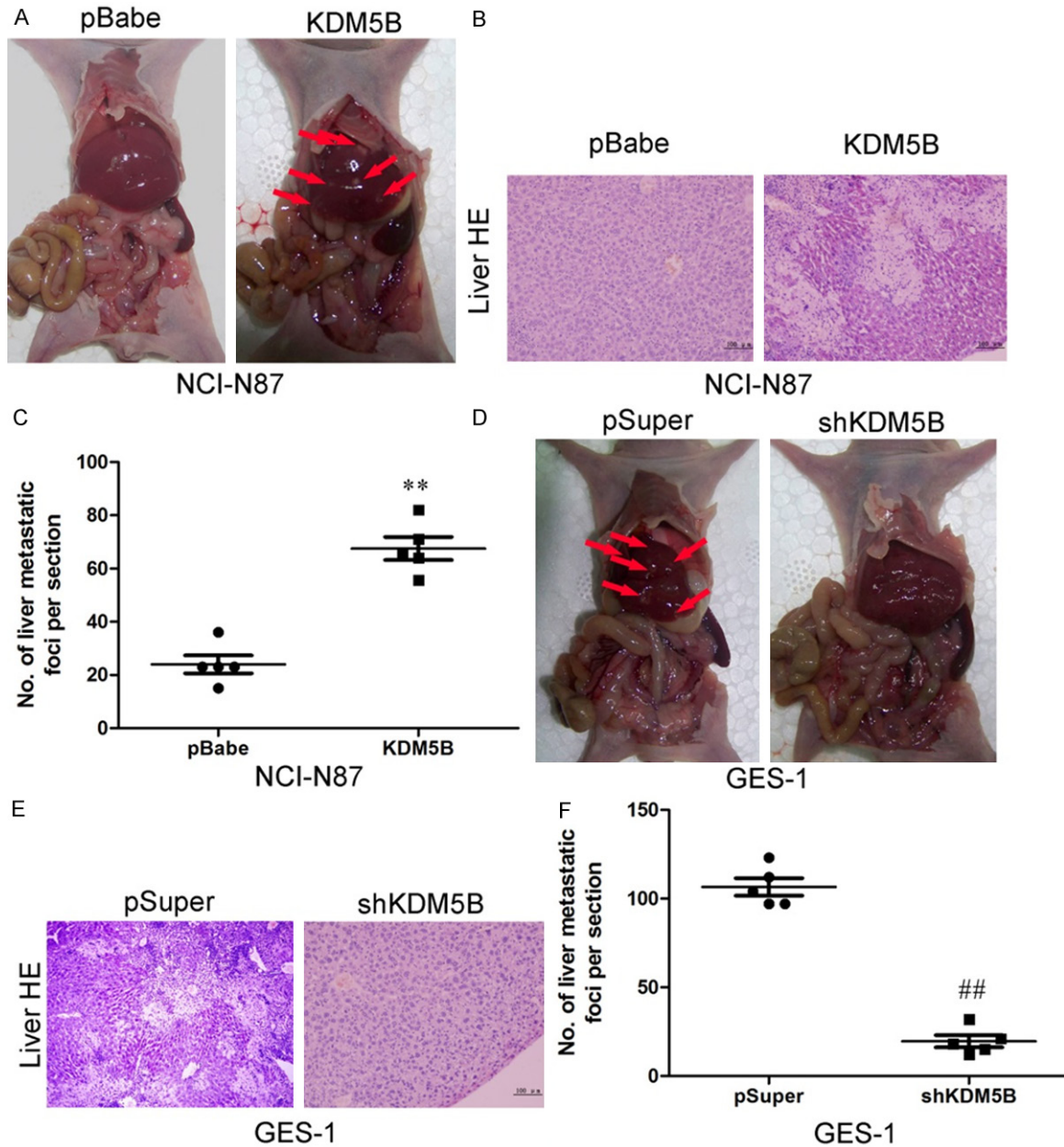


Figure 7. KDM5B promoted gastric cancer cell metastasis in vivo. A. Representative livers that were harvested from the mice that had been injected in the lateral tail veins in KDM5B-overexpressing NCI-N87 and its control cells. B. KDM5B-overexpressing NCI-N87 and its control cells representative hematoxylin and eosin staining of liver sections (magnification x40). C. The numbers of metastatic foci per section in liver of individual mouse with injection of KDM5B-overexpressing NCI-N87 and its control cells. D. Representative livers that were harvested from the mice that had been injected in the lateral tail veins in KDM5B-silenced GES-1 and its control cells. E. KDM5B-silenced GES-1 and its control cells representative hematoxylin and eosin staining of liver sections (magnification x40). F. The numbers of metastatic foci per section in liver of individual mouse with injection of KDM5B-silenced GES-1 and its control cells. ** $P < 0.01$; ## $P < 0.01$.

tion, KDM5B knockdown in GES-1 cells significantly inhibited cell migration and invasion (Figure 5C and 5D). These results indicated that KDM5B significantly inhibited the invasion and migration of gastric cancer cells.

KDM5B enhanced tumorigenesis and metastasis in vivo

To explore the effects of KDM5B on tumorigenesis in vivo, different cell lines were injected

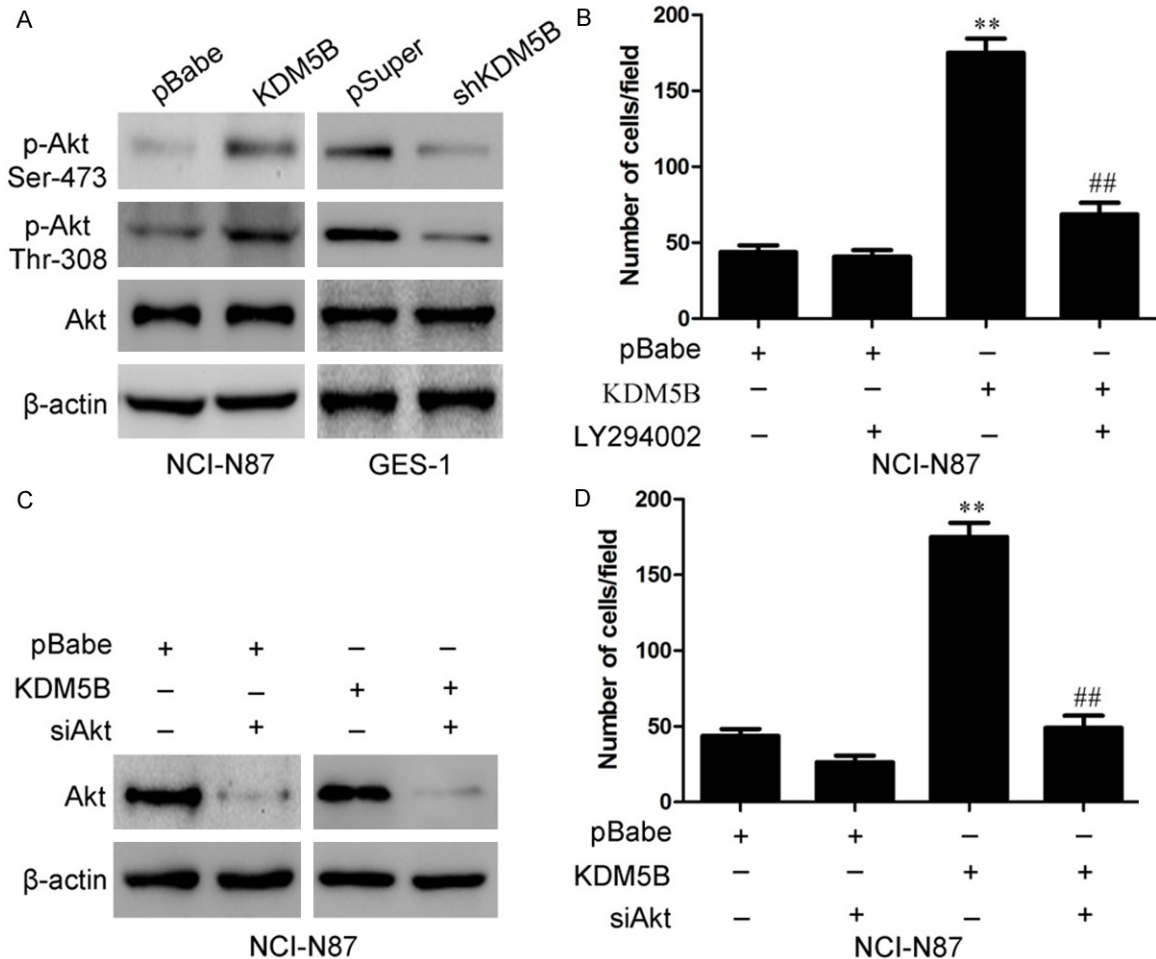


Figure 8. The effects of KDM5B on the Akt pathway. A. The expression of Akt and the phosphorylation of Akt at Ser 473 and Thr 308 in KDM5B-transfected cells were examined using western blotting. β -actin was used as a loading control. B. The summary graphs show the migration ability of KDM5B overexpressed NCI-N87 and its control cells after the cells had been pretreated with the Akt inhibitor LY294002 (100 nM). C. The transfection efficiency of Akt siRNA 24 h after transfection was measured using western blot analyses in NCI-N87 cells. β -actin was used as a loading control. D. The summary graphs show the migration ability of KDM5B overexpressed NCI-N87 and its control cells after the cells had been transfected with Akt siRNA and its negative control. The data represent the mean number of cells per field and are presented as the means \pm S.D. ** $P < 0.01$ vs Control group; ## $P < 0.01$ vs shKDM5B group.

subcutaneously into the flanks of nude mice. The diameters of the tumors were measured every 3 days. We found that the mice that had been injected with the KDM5B-overexpressing NCI-N87 cells formed tumors on the ninth day, while the mice that had been injected with control NCI-N87 cells did not form tumors until the twelfth day. The tumors in the KDM5B overexpression group grew very quickly compared with those in the mice that had been injected with the control cells during the subsequent days (Figure 6A). Similar results were observed in the GES-1 cells. We found that the control cells formed tumors earlier and that the tumor

volumes were much larger in those that were formed from the control cells than in those that were formed from the KDM5B knockdown cells (Figure 6D). As shown in Figure 6B and 6C, the average tumor volume and weight of NCI-N87 control was much lower than KDM5B-overexpressing NCI-N87 cells. Similar with these, the control group was dramatically larger than the shKDM5B group in GES-1 cells (Figure 6E and 6F). We next examined the KDM5B and PCNA by immunohistochemistry staining in tumor tissues. As shown in Figure 6G, we found that the overexpression of KDM5B in NCI-N87 cells significantly upregulated PCNA staining.

Also knockdown of KDM5B in GES-1 cells dramatically downregulated the staining of PCNA. These results suggested that KDM5B inhibits gastric cancer cell xenograft formation and growth in vivo.

We then investigated the functional relevance of KDM5B for metastasis in vivo. NCI-N87-KDM5B, GES-1-shKDM5B and their corresponding control cells were injected into nude mice through the tail vein. KDM5B overexpression dramatically increased the number of metastatic tumors in liver of each mouse (**Figure 7A-C**). Silencing KDM5B in GES-1 cells inhibited metastatic behavior, in terms of the number of metastatic tumors in the liver of each mouse (**Figure 7D-F**). Therefore, the in vivo results further demonstrate the critical role of KDM5B in gastric cancer metastasis.

KDM5B promoted tumor metastasis via activation of the Akt pathway

The PI3K/Akt pathway has important roles in the proliferation, migration and invasion of various cancer types, including gastric cancer [19]. Thus, we determined whether the Akt pathway was involved in KDM5B-mediated tumor metastasis. We evaluated the effects of KDM5B on the Akt pathway in NCI-N87 and GES-1 cells by measuring the phosphorylation profile of Akt at Ser 473 and Thr 308. As shown in **Figure 8A**, upregulation of KDM5B significantly activated the phosphorylation of Akt Ser 473, Akt Thr 308 and knockdown of KDM5B dramatically inhibited the Akt Ser 473 and Thr 308.

To test whether the Akt pathway was involved in the KDM5B induced metastatic function, we pretreated the NCI-N87 cells with LY294002 for 1 h, after which point we detected the migration ability of the control and KDM5B overexpressed cells using a transwell assay. We found that the number of KDM5B overexpressed cells that had migrated was significantly decreased after LY294002 treatment, while there was only a slight decrease in the number of control NCI-N87 cells that had migrated (**Figure 8B**). We further tested the role of Akt in KDM5B-induced migration by knocking down Akt expression using siRNA. The Akt knockdown efficiency was detected using western blotting at 24 h after transfection (**Figure 8C**). As shown in **Figure 8D**, the migration ability that was induced by KDM5B was obviously attenuated

following Akt knockdown using siRNA. These results confirmed that the Akt pathway was involved in KDM5B-mediated metastasis in gastric cancer cells.

Discussion

Gastric cancer is one of the most common carcinomas in the world. Although its incidence has been slowly declining, it remains the second leading cause of cancer death worldwide [20]. The 5-year survival rate for patients with gastric cancer is only about 20%. The high mortality rates in patients with gastric cancer are associated with metastatic spread of cancer cells from the stomach to common sites such as the liver and peritoneum [21]. Metastasis is the result of several sequential steps including proliferation, invasion into adjacent tissues, detachment of tumor cells from primary tumor, migration into lymph nodes and blood vessels, adhesion and survival in the circulation, and extravasation into the target organ, where, again, proliferation occurs [22, 23]. Molecules involved in each step of the metastasis process are potential prognostic and therapeutic markers [18, 24]. In the present manuscript, we identified KDM5B as a candidate target gene for gastric cancer growth and metastasis.

A growing body of evidence indicates that overexpression or mutations of histone methyltransferases and demethylases have been linked to the development of many human cancers [25-27]. Histone H3K27 methyltransferase, EZH2, and H3K4 demethylases LSD1 and KDM5B have been thought to play important roles not only in tumor initiation, but also in tumor progression, since overexpression of these genes has been reported in many types of malignant tumors [26]. Previous papers revealed that upregulation of KDM5B enhanced cell migration, cell invasion and EMT to promote malignant progression of lung cancer cells [28]. However, the role of KDM5B in gastric cancer carcinogenesis remains unclear. To confirm the oncogene function of KDM5B, we first examined the levels of KDM5B in gastric cancer samples and matched normal gastric tissue samples. We found that KDM5B was significantly overexpressed in gastric cancers, which suggested that KDM5B was a candidate oncogene in gastric cancer. To further explore the role of KDM5B in gastric cancer, we transfected gastric cancer cells either to ectopically

express KDM5B or to inhibit its expression using RNA interference. Overexpression of KDM5B in vitro significantly enhanced the proliferation, migration and invasion of gastric cancer cells, while knockdown of KDM5B inhibited cell growth and mobility. Our in vivo experiments also demonstrated that KDM5B markedly promoted tumorigenesis and metastasis to the liver. These data further supported the oncogene role of KDM5B in gastric cancer.

The activated Akt pathway has been demonstrated to have an essential role in gastric cancer proliferation, motility and invasion, and MMP-2 and MMP-9 have been shown to enhance cancer migration and invasion via regulation of the Akt pathway [29-31]. Therefore, we investigated whether KDM5B promoted tumorigenesis and metastasis in gastric cancer via activation of the Akt pathway. Our results indicated that the levels of p-Akt were significantly increased in KDM5B-overexpressing cells and p-Akt were downregulated in KDM5B knockdown cells. When we pretreated the KDM5B overexpressed cells with Akt inhibitor or Akt siRNA, the increased migration ability of KDM5B overexpressed cells was also inhibited. All of these data revealed that KDM5B promotes the migration activities of gastric cancer cells partly via activation of the Akt pathways. However, how KDM5B might regulate the Akt pathway is still need further study.

Conclusions

In conclusion, we found that KDM5B expression was generally higher in gastric cancer lesions compared with matched non-tumor tissues. Our data demonstrate that KDM5B has a vital function in promoting cell mobility, which is at least partially controlled by the Akt pathway. Thus, we propose that the candidate tumor oncogene KDM5B, together with the Akt signaling pathway, maybe an effective novel therapeutic target in the management of gastric cancer.

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Disclosure of conflict of interest

The authors have no competing interests to disclose.

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